Week 5 Notebook

June 29, 2015 - July 2, 2015

June 29, 2015

Kayla/Julie

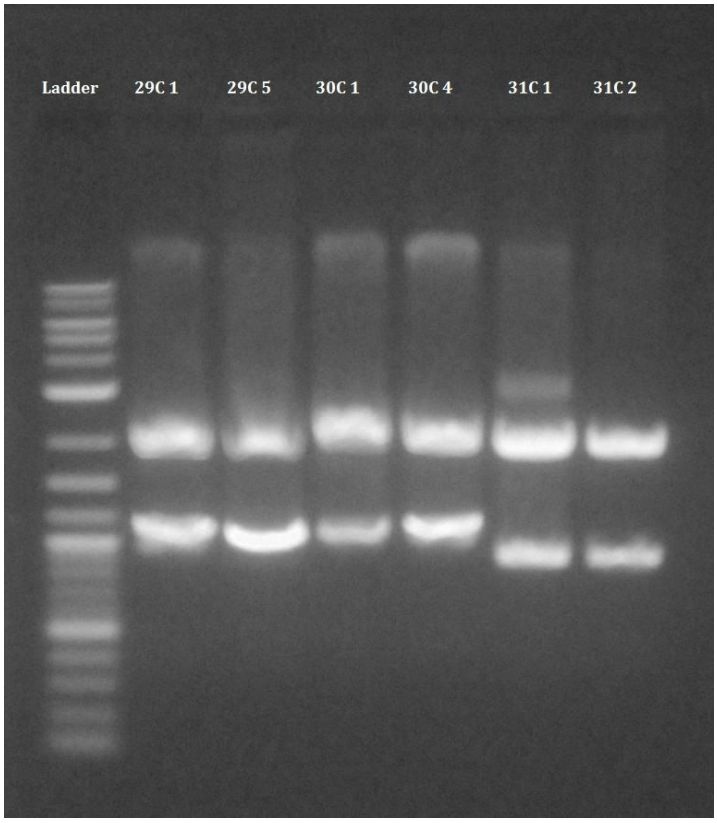
- Minipreps of 15 liquid cultures (29C 1-5, 30C 1-5, and 31C 1-5)
    - Measured absorbances at 260 nm and calculated DNA concentration

Sample	DNA Concentration (ng/μL)
29C 1	1593
29C 2	372
29C 3	360
29C 4	642
29C 5	990
30C 1	423
30C 2	Did not pass glow screening
30C 3	297
30C 4	309
30C 5	57
31C 1	606
31C 2	750
31C 3	321
31C 4	108
31C 5	Negative absorbance

- Test digest of 29C1, 29C5, 30C3, 30C5, 31C1, and 31C2 with XbaI and SpeI
    - 50μL reaction
        - 10 μL miniprep
        - 5 μL buffer
        - 2 μL XbaI

- 2 µL SpeI
- 31 µL H2O

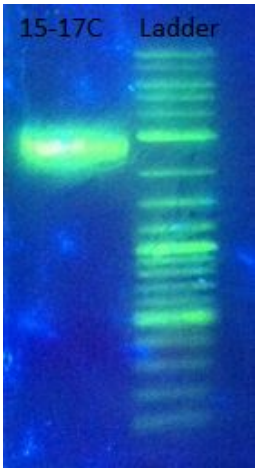
- Ran on 1.0% agarose gel at 90V for 45 minutes
  - all digests appeared to have an insert around 1 kb in length
  - will 29C, 30C, and 31C minipreps with highest DNA concentration for sequencing tomorrow



Chloe/Charlotte

The 15-7C miniprep was digested with E and P to yield a new plasmid backbone to use for ligations. The entire digestion was run in a gel.

Unfortunately, the gel showed that the restriction digest of 15-7C was not successful, as there was only one band on the gel, pictured below. Because there was no more P available, another digest could not be prepared.



More Cam plates were poured.

Content and graphics for the wiki were worked on.

Dave/Eddie

- Made a glycerol stock and conducted a miniprep of the RiAFP (15-21C) grown over the weekend.
- Found the concentration of the miniprep:

	Absorbance @260	Absorbance @280	Concentration (ug/ul)
15-21C	0.0073	0.0056	120

- Sent a sample away for sequencing.
- Digested remaining 15-17C miniprep (30 ul with 5 ul buffer, 2 ul each S and P, 11 ul water) and gel purified with the following wells:
    - 1 Nothing (Well was tilted)
    - 2 Ladder
    - 3 15-17C
    - 4 15-17C
- Picked 8 colonies from the 15-17C plate to grow up liquid culture. A liquid culture was also prepared for 15-21C from the glycerol stock.
- Ligated, transformed, and plated each of the four new AFPs with the new 15-17C miniprep according to the table below:

		Vector (ul)	Insert (ul)	Buffer (ul)	Ligase (ul)	Water (ul)	Total (ul)
1	1 ul	1	0	2	1	16	20

	Control						
2	2 ul Control	2	0	2	1	15	20
3	GaAFP	1	2	2	1	14	20
4	GaAFP	2	2	2	1	13	20
5	GaAFP	1	5	2	1	11	20
6	GaAFP	2	5	2	1	10	20
7	RiAFP	1	2	2	1	14	20
8	RiAFP	2	2	2	1	13	20
9	RiAFP	1	5	2	1	11	20
10	RiAFP	2	5	2	1	10	20
11	BrAFP	1	2	2	1	14	20
12	BrAFP	2	2	2	1	13	20
13	BrAFP	1	5	2	1	11	20
14	BrAFP	2	5	2	1	10	20
15	TmAFP	1	2	2	1	14	20
16	TmAFP	2	2	2	1	13	20
17	TmAFP	1	5	2	1	11	20
18	TmAFP	2	5	2	1	10	20

June 30, 2015

Kayla/Julie

- Prepared 30% acetic acid solution
- Pipetted 200 µL of 30% acetic acid into wells used in the 6/9, 6/16, and 6/26 biofilm plates to dissolve crystal violet
- Transferred 125µL from stained wells into flat bottom 96-well plates
- Measured OD_{595nm} of all wells in flat bottom plates using a plate reader
    - initial readings
        - Blank=second table, wells A10-D10

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.038	0.044	0.039	0.039	0.04	0.048	0.039	0.041	0.096	0.039	0.052	0.044	595
B	0.039	0.045	0.039	0.039	0.038	0.045	0.043	0.041	0.101	0.038	0.053	0.048	595
C	0.038	0.049	0.039	0.038	0.038	0.04	0.043	0.044	0.087	0.04	0.057	0.04	595
D	0.038	0.05	0.039	0.039	0.042	0.039	0.044	0.043	0.084	0.041	0.06	0.038	595
E	0.043	0.092	0.039	0.048	0.042	0.044	0.042	0.042	0.086	0.039	0.055	0.037	595
F	0.043	0.088	0.04	0.042	0.045	0.05	0.043	0.045	0.085	0.039	0.065	0.037	595
G	0.042	0.092	0.037	0.042	0.045	0.046	0.043	0.044	0.084	0.04	0.059	0.038	595
H	0.041	0.078	0.037	0.048	0.046	0.046	0.046	0.045	0.074	0.038	0.053	0.038	595
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
A	0.072	0.055	0.378	0.059	0.045	0.291	0.065	0.051	0.4	0.034	0.026	0.031	595
B	0.075	0.054	0.283	0.067	0.061	0.255	0.083	0.05	0.283	0.034	0.03	0.03	595
C	0.082	0.053	0.359	0.073	0.057	0.254	0.109	0.052	0.287	0.035	0.031	0.03	595
D	0.09	0.048	0.25	0.102	0.05	0.221	0.09	0.048	0.278	0.034	0.03	0.034	595
E	0.03	0.047	0.455	0.092	0.048	0.193	0.117	0.047	0.315	0.032	0.029	0.03	595
F	0.121	0.064	0.394	0.084	0.051	0.146	0.076	0.048	0.228	0.03	0.03	0.03	595
G	0.125	0.066	0.273	0.101	0.059	0.317	0.074	0.048	0.124	0.03	0.031	0.029	595
H	0.11	0.064	0.378	0.094	0.052	0.188	0.086	0.047	0.228	0.03	0.031	0.031	595

- Readings sorted and averaged, with blank subtracted

	Plate1	Plate 2 T1	Plate 2 T2	Plate 3 T1	Plate 3 T2
DH5α LB	0.03825	0.03875	0.045		
DH5α Difco		0.0395	0.0445		
DH5α M9	0.00775	0.0085	0.012		
EMG2:Kλ LB	0.0125	0.00775	0.009	0.07975	0.0965
EMG2:Kλ Difco		0.00775	0.0095	0.0525	0.06025
EMG2:Kλ M9	0.053	0.0575	0.04775	0.283	0.30225
ML308 LB		0.005	0.005		
ML308 Difco		0.021	0.02175		
ML308 M9		0.008	0.00625		
BclA-YFP LB				0.04075	0.049
BclA-YFP Difco				0.01875	0.0195
BclA-YFP M9				0.22075	0.19625
GFP LB				0.05225	0.06525
GFP Difco				0.01575	0.01475
GFP M9				0.2775	0.25625

- Readings normalized to the highest OD for EMG2K:λ in M9 (strongest biofilm)

	Plate1	Plate 2 T1	Plate 2 T2	Plate 3 T1	Plate 3 T2
DH5α LB	0.721698	0.673913	0.942408		
DH5α Difco		0.686957	0.931937		
DH5α M9	0.146226	0.147826	0.251309		
EMG2:Kλ LB	0.235849	0.134783	0.188482	0.281802	0.319272
EMG2:Kλ Difco		0.134783	0.198953	0.185512	0.199338
EMG2:Kλ M9	1	1	1	1	1
ML308 LB		0.086957	0.104712		
ML308 Difco		0.365217	0.455497		
ML308 M9		0.13913	0.13089		
BclA-YFP LB				0.143993	0.162117
BclA-YFP Difco				0.066254	0.064516
BclA-YFP M9				0.780035	0.649297
GFP LB				0.184629	0.215881
GFP Difco				0.055654	0.048801
GFP M9				0.980565	0.847808

Chloe/Charlotte

A restriction digest was prepared of both 15-6C and 15-7C with E and P to attempt to make more vector again. The resulting digests were run on a gel (pictured below). The gel showed that the digest was unsuccessful again, showing one only band for each product.

From there, we switched back to the linearized plasmid backbone and tried to digest it again, this time with 10 uL of backbone instead of the recommended 4 uL. The digest was incubated at 37℃ for an hour, then heat killed at 80℃ for 20 minutes.
The GFP and BclA inserts were purified again, eluted with 20 ul of water rather than the typical 30 ul. The plasmid backbone was also purified. Ligations were performed with the following ratios of vector to insert:

- 2 ul : 0 ul (control)
- 2 ul : 5 ul BclA

- 5 ul : 5 ul BclA
- 2 ul : 5 ul GFP
- 5 ul : 5 ul GFP

A transformation of the ligations was performed, and the result was plated and left overnight.

A freeze survival assay was prepared with sequence-confirmed 15-21C (J23119+RiAFP) and 15-17C (plain J23119). 100 uL of each culture was used for a serial dilution up to 10-6, repeated 3 times. The 10-6 dilutions were all plate and left to incubate at 37℃ overnight. 500 uL of each culture was stored at either -20℃ or -80℃ and left to slow-freeze overnight.

Dave/Eddie

- Made a glycerol stock and miniprepped 15-17C, and miniprepped 15-21C samples.
- Specd the samples, table below:

Concentration

	@ 260nm	@ 280nm	Concentration (ug/ul)
15-17C 1	0.0320	0.0189	528
15-17C 2	0.0154	0.0078	254
15-17C 3	0.0024	-0.0024	40
15-17C 4	0.0213	0.0103	351
15-17C 5	0.0369	0.0214	609
15-17C 6	0.0390	0.0239	644
15-17C 7	0.0469	0.0340	774
15-17C 8	0.0133	0.0068	219
15-21C	0.0439	0.0261	724

9ul of DNA used with 291ul H2O

- Picked two colonies of GaAFP and three colonies each of BrAFP and TmAFP and prepared liquid cultures.

Colonies on ligation plates

		Vector (ul)	Insert (ul)	Colonies	Picked
1	1 ul Control	1	0	2	N
2	2 ul Control	2	0	0	N
3	GaAFP	1	2	1	Y 1
4	GaAFP	2	2	0	N

5	GaAFP	1	5	1	Y 1
6	GaAFP	2	5	0	N
7	RiAFP	1	2	3	N
8	RiAFP	2	2	0	N
9	RiAFP	1	5	2	N
10	RiAFP	2	5	4	N
11	BrAFP	1	2	2	Y 2
12	BrAFP	2	2	0	N
13	BrAFP	1	5	0	N
14	BrAFP	2	5	3	Y 1
15	TmAFP	1	2	4	Y 1
16	TmAFP	2	2	3	Y 1
17	TmAFP	1	5	1	Y 1
18	TmAFP	2	5	0	N

- Digested 15-17C 1 with S and P for two hours in a 50 ul total reaction.

July 1, 2015

Kayla/Julie

- Analyzed interlab sequencing results
    - J23101+I13504 (29C) and J23106+I13504 (30C) are sequence confirmed
    - 31C had a mutation in the promoter region
        - picked 3 new colonies off of the 31C transformation plate and grew in LB broth overnight
- Grew a 50 mL overnight liquid culture of pDC1

Chloe/Charlotte

No colonies were found on the GFP and BclA transformation plates from yesterday. Plates were returned to the 37 C incubator.

Colonies of Stationary Phase Growth *E. coli* Plates (Before Freeze)			
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
15-17C 10-6 (1)	286	15-21C 10-6 (1)	121
34C 10-6 (2)	91	15-21C 10-6 (2)	56
34C 10-6 (3)	795	15-21C 10-6 (3)	345
Average:	390.67	**Average:**	174

Transformation plates were re-checked and one colony was found on the BclA 5:5 plate. An overnight liquid culture was prepared.

Dave/Eddie

- Miniprepped the eight cultures grown overnight, setting aside 500 ul to make glycerol stocks of the good ones once we've run a gel. Concentrations are as follows:

	@260	@280	Concentration
15-20C 1	0.0095	0.0069	157
15-20C 2	0.0137	0.0099	226
15-22C 1	0.0176	0.0119	290
15-22C 2	0.0206	0.0144	340
15-22C 3	0.0151	0.0098	249
15-23C 1	0.0293	0.0217	483
15-23C 2	0.0214	0.0171	353
15-23C 3	0.0249	0.0188	411

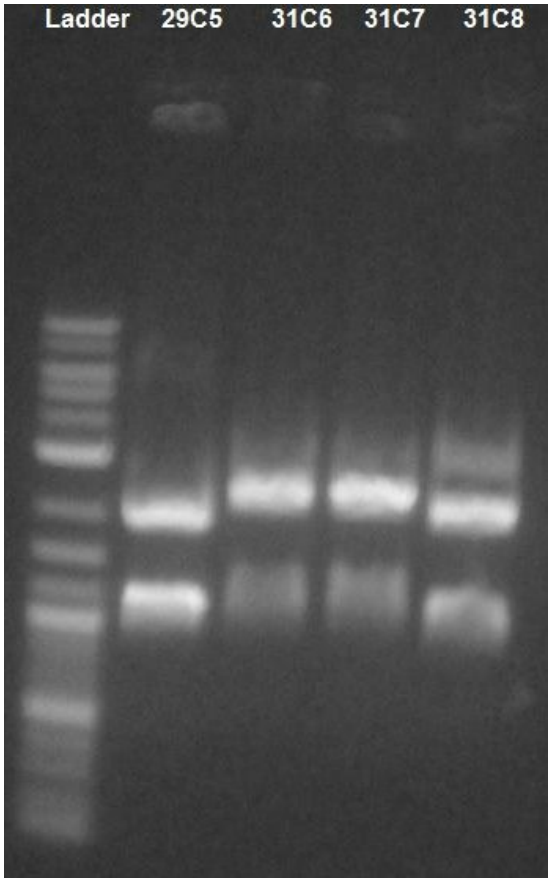
- Ran a gel and purified the 15-17C digest from yesterday.
- Ran a test digest of the eight AFP cultures and ran a gel:
    - 1 Ladder
    - 2 15-20C 1
    - 3 15-20C 2
    - 4 15-22C 1
    - 5 15-22C 2

- 6 15-22C 3
- 7 15-23C 1
- 8 15-23C 2
- 9 15-23C 3

July 2, 2015

Kayla/Julie

- Miniprep of 31C 6, 31C 7, and 31C 8
  - test digested with XbaI and PstI
    - 15 µL reaction
  - ran on 1.0% agarose gel at 100V for 40 minutes



  - insert appeared to be present
    - same size as GFP insert in 29C
- Prepared competent cells from pDC1 and pDC1+GFP

Chloe/Charlotte

PCR products (GFP and BclA inserts) were purified and placed in -20 C for storage.

A miniprep of the BclA-transformed culture was prepared.

Colonies of Stationary Phase Growth *E. coli* Plates After Freeze)							
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
-20 15-17C 10-6 (1)	266	-80 15-17C 10-6 (1)	306	-20 15-21C 10-6 (1)	196	-80 15-21C 10-6 (1)	99
-20 15-17C 10-6 (2)	376	-80 15-17C 10-6 (2)	191	-20 15-21C 10-6 (2)	715	-80 15-21C 10-6 (2)	109
-20 15-17C 10-6 (3)	776	-80 15-17C 10-6 (3)	29	-20 15-21C 10-6 (3)	440	-80 15-21C 10-6 (3)	85
Average:	472.67	**Average:**	175.33	**Average:**	450.3	**Average:**	97.67
Survival	1.21	**Survival**	0.45	**Survival**	2.59	**Survival**	0.56

Dave/Eddie

We have to redo the test digest with X and P, E and P, and no digest

- 15-13C was digested under the following conditions:
  - X and P
  - E and P
  - No digest
- The digest was run for 2 hours at 37C
- Gel was run with the following bands
  - Ladder
  - X and P
  - E and P
  - No digest
  - X and P
  - E and P
  - No digest
- The gel showed that both enzymes will cut , provided they have 2 hours to do so.